



Pharmacognostical, preliminary phytochemical analysis, phenetics of *Artabotrys hexapetalus* (L.f) Bhandari leaves

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ABSTRACT :

Introduction: *Artabotrys hexapetalus* (L.f.) Bhandari (Annonaceae) is a medicinal plant traditionally used in India and other Asian countries for the treatment of malaria, cholera, fungal infections, rheumatism and kidney disorders. The present study was undertaken to establish the pharmacognostical and phytochemical characteristics of the leaves of *Artabotrys hexapetalus* and to provide suitable parameters for its identification and quality evaluation.

Materials and methods: Fresh leaves of *Artabotrys hexapetalus* were collected from Ethappur village, Salem district in Tamil Nadu, India in the month of January 2026. The plant sample was authenticated by Dr. D. Stephen, Assistant Professor (retd), Department of Botany, American college, Madurai-625 020. Macroscopical, microscopical evaluation, powder microscopical, quantitative microscopical and histochemical studies were carried out. Limit of detection (LOD) and limit of quantitation (LOQ) were determined. Physicochemical parameters, fluorescence behaviour, phenetics, preliminary phytochemical screening and quantitative estimation of total phenolic, tannin and flavonoid contents were performed using standard methods.

Results and discussion: Leaves were simple, alternate, lanceolate and glabrous, with an acuminate apex, cuneate base, entire margin and reticulate venation. Transverse sections showed a dorsiventral lamina with single-layered epidermis, uniseriate palisade and a single collateral vascular bundle in the midrib, while the petiole showed three collateral bundles with prismatic crystals. Powder microscopy revealed paracytic stomata, stone cells, fibres, vessels and prismatic crystals, with leaf constants including vein islet number 35, stomatal index 15 and palisade ratio 1–7. Loss on drying was 16.18%, total ash 12.19%, with lead absent. Extract yield was 23.34% w/w, showing alkaloids, phenols, tannins, flavonoids, terpenoids and proteins. Total phenolic, flavonoid and tannin contents were 616.7 mg GAE/g, 550 mg QE/g and 520 mg TAE/g, with an LOD/LOQ of 18.15 & 55 µg/ml. Phenetic analysis gave a matching coefficient of 76% with related species.

Conclusion: The present study establishes reliable pharmacognostical and phytochemical standards for *Artabotrys hexapetalus* leaves. The macroscopic, microscopic and powder characters, together with the physicochemical parameters and absence of lead, serve as dependable markers for authentication of the drug. Phytochemical screening confirmed the presence of alkaloids, phenols, tannins and flavonoids, with the extract showing notable phenolic, flavonoid and tannin contents. Phenetic analysis further revealed close similarity of *A. hexapetalus* with related species, thereby establishing useful reference standards for its identification and quality control.

Key words: Annonaceae, *Artabotrys hexapetalus*, pharmacognostical, phenetics, phytochemical, qualitative and quantitative analysis of phytoconstituents.

INTRODUCTION

Artabotrys hexapetalus (L.f.) Bhandari belongs to Annonaceae, a large erect lactiferous shrub. It is indigenous shrub to Southern India, Sri Lanka and widely grown in southern China, Philippines, Indo-china and Java (1). Its roots preparations are used by indigenous communities of Tiruvannamalai, Tamil Nadu, India and its stem preparations are used in Kandal province, Cambodia to treat malaria (2,3). People of Assam, used fruits to treat fungal infections. Throughout Southern India, the flowers are valued for the treatment of bad breath, biliousness, bladder disorders, blood and heart diseases, headache, itching, leucoderma, excessive sweating, thirst and vomiting (4). People of Bangalore, Karnataka, uses the plant as cardiac stimulant, muscle relaxant and uterine stimulant (5). Tribes of Malay archipelago and Hazaribagh, Jharkhand uses leaves for the treatment of chorea (6,7). In Hainan, Southern China, the toasted bark is traditionally used as a remedy for gastrointestinal disorders (8). In Korogwe, Tanzania, a decoction prepared from the roots and leaves, often in combination with the roots of *Uvaria leptocladon*, is administered to alleviate abdominal and kidney pain (9). In Thailand, the flowers are traditionally used as a cardiotonic (10). Phytochemical review revealed the presence of carbohydrates, proteins, alkaloids, flavonoids, terpenoids and tannins (11). It possesses significant antimalarial (12), antioxidant (13), antifertility, antimicrobial (14), hepatoprotective (15), anti-inflammatory and antipyretic (16), anthelmintic and cytotoxic activities (17). The present study is to examine *Artabotrys hexapetalus* sourced from a

distinct habitat offering fresh insight into the plant's Pharmacognostical, Physico-chemical parameters, Qualitative analysis of phytoconstituents present in *Artabotrys hexapetalus* leaf and quantitative estimation of gallic acid, quercetin and tannic acid was determined along with initiation of phenetic studies. The present research was investigated to draw pharmacopoeial parameters for this medicinal plant.

MATERIALS AND METHODS

Collection of leaf and authentication

The leaves of *Artabotrys hexapetalus* were collected from, Ethappur village, Salem district in Tamil Nadu, India in the month of January 2026. The plant sample was authenticated by Dr. D.Stephen, Assistant Professor (ret'd), Department of Botany, American college, Madurai-625 002.

Pharmacognostical evaluation

Morphology

Morphological characters such as size, shape, apex, margin, venation, base, petiole, surface and colour of leaves of *Artabotrys hexapetalus* were studied (18).

Microscopical study

Thin fresh and transverse sections of the leaf, including the petiole and midrib, of *Artabotrys hexapetalus* were prepared. The sections were stained using 0.8% safranin and 0.5% Astra blue and subsequently observed under the microscope (19).

Quantitative microscopy

It was performed using square pieces of leaf taken from the region between the midrib and the margin. These samples were decolourised and examined under a microscope to assess vein islet number, vein termination and palisade ratio. For stomatal index analysis, the upper and lower epidermal layers were carefully peeled from separate leaf pieces, mounted in glycerin water on a slide and observed microscopically to determine stomatal index and stomatal type (19).

Preparation of leaves Powder

The fresh matured leaves of *Artabotrys hexapetalus* were collected, washed with water and dried. The dried leaves were made into a fine powder.

Transverse Section

Sample was preserved in fixative FAA for more than 48 hr, the preserved specimens were cut into thin transverse section using a sharp blade and the sections were stained with 0.8% safranin and 0.5% Astra blue. Transverse sections were photographed using Nikon ECLIPSE E200 trinocular microscope attached with zeiss, AxioCam Erc5s digital camera under bright field light. Magnifications were indicated by scale bar (20).

Powder Microscopy

A pinch of the powdered sample was mounted on a microscopic slide with a drop of 50% glycerol after clearing with saturated solution of chloral hydrate. Sample was treated with iodine solution to confirm the presence of starch grains. Characters were observed using Nikon ECLIPSE E200 trinocular microscope attached with Zeiss ERc5s digital camera under bright field light. Photomicrographs of diagnostic characters were captured and documented (21).

Behaviour of Powder with various chemical reagents

A small quantity of leaf powder was transferred into a test tube, to which 1–2 drops of freshly prepared reagents were added. The resulting colour changes were observed under visible light as well as under UV at 254 nm and 365 nm, following standard procedure (22).

Physicochemical parameter

The powder was subjected to physiochemical parameters such as loss on drying, ash value and extractive value with different solvents in increasing order of polarity as per standard procedures recommended by Ayurvedic pharmacopoeia of India (23). The ash was subjected to inorganic elements identification as per Atherden (24) and heavy metals analysis of lead were performed as per I.P 1985 (25).

Preparation of hydroalcoholic extract of *Artabotrys hexapetalus* (HAEAH₁)

Collect leave part of *Artabotrys hexapetalus*, dried and make a coarse powder .The powder will soak into 7:3 ratio of ethanol and water respectively for 72 hrs of maceration. After maceration filter the solution and make concentrate of extract. pH was determined.

Preliminary phytochemical screening

Preliminary phytochemical screening were carried out by using different reagents for identification of presence of phytoconstituents as per standard procedures (26).

Quantitative estimation of phytoconstituents

Limit of Detection (LOD)

LOD of an individual analytical procedure is the lowest amount of an analyte in a sample that can be detected but not necessarily quantified.

Limit of Quantitation (LOQ)

LOQ of an individual analytical procedure is the lowest amount of analyte in a sample that can be detected and quantified.

Procedure

Fresh extract of *Artabotrys hexapetalus* was prepared and scanned across the UV range were recorded. Subsequently, extract samples at concentrations ranging from 20 to 100 µg/ml were prepared and analyzed using UV spectrophotometer (Shimadzu-UV 1800) at the previously identified wavelength. The LOD and LOQ values were calculated based on the standard deviation of the response and the slope obtained from the calibration curve of *Artabotrys hexapetalus* extract (27).

Determination of Gallic acid equivalent in *Artabotrys hexapetalus*

Procedure

A series of calibrated 10ml volumetric flask is taken and standard solution (gallic acid) and extract solution of various concentrations (20µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml) is taken. To each of this solution add 5ml of distilled water and 0.5ml of Folin's Ciocalteu's reagent is added, mixed and shaken. After 5 minutes, 1ml of 10% sodium carbonate solution is added and the volume is made up to 10 ml with distilled water. It is allowed to incubate for 2 hours at room temperature. Intense blue colour is developed. The reaction mixture without sample is used as blank. After

incubation, absorbance is measured at 725nm using UV spectrophotometer and the mean values will be recorded. The calibration curve will be plotted using standard gallic acid. Total phenolic content of extract is expressed in terms of mg of gallic acid equivalent per gm of extract (mg GAE/g) (28).

Determination of Quercetin equivalent in *Artabotrys hexapetalus*

Procedure

1ml of extract, 0.1 ml of aluminium chloride solution, 0.1 ml of potassium acetate solution and 2.8 ml of ethanol were added and the final volume was then made up to 5 ml with distilled water. After 20 mins the absorbance was measured at 415 nm. A calibration curve was constructed by plotting absorbance reading of quercetin at different concentrations (20, 40, 60, 80, 100 µg/ml). The sample without aluminium chloride was used as a blank. The total flavonoid content in the extract was expressed as milligrams of quercetin equivalent per gram of extract (28).

Determination of Tannic acid equivalent in *Artabotrys hexapetalus*

Procedure

Prepare various concentration of hydroalcoholic extract of *Artabotrys hexapetalus* into test tubes. To this, 0.5 ml of Folin- Denis reagent and 0.8 ml of distilled water was added. The tubes were kept aside for 15mins. To this, 1ml of 10% sodium carbonate solution was added and the remaining volume was made up with 7.5 ml of distilled water. Carbonate solution was added and the remaining volume was made up with 7.5 ml of distilled water. Then the tubes were shaken and the absorbance was recorded at 700nm after 30min. Tannic acid, used as a standard was taken at different concentration 20, 40, 60, 80, 100 µg/ml in different test tubes and the procedure adopted above was followed. The calibration curve for tannic acid was plotted using concentration versus absorbance. A linear regression equation was calculated and the equation was used to calculate the amount of total tannins as tannic acid equivalent. Total Tannin content of *Artabotrys hexapetalus* leaf extract is expressed in terms of mg of Tannic acid equivalent per gm of extract (mg TAE/g) (29).

Phenetics

Numerical taxonomy of plant derived by using five characters such as leaf shape, venation, apex, texture and margin (30).

RESULTS AND DISCUSSION

Morphological study

The leaves are simple, alternately arranged in two vertical rows and attached by short petioles (3–8 mm) without stipules. Their shape ranges from oblong-lanceolate to elliptic, measuring 6–10 mm in length, with a narrowly pointed tip and a base that is either sharp or wedge-shaped. Both leaf surfaces are smooth and somewhat leathery, featuring a flat mid vein on the upper side and a prominent one beneath, along with a pattern of flat lateral veins. When dried, the leaves take on a pale green color.



Fig 1: Habitat of *A. hexapetalus*



Fig 1a: Dorsal surface



Fig 1b: Ventral surface

Table 1: Morphological characters of leaves of *Artabotrys hexapetalus*

S.No	Characters	Observation
1	Colour	Green
2	Odour	Pleasant
3	Taste	Slightly bitter
4	Leaf type	Simple
5	Shape	Lanceolate
6	Arrangement	Alternate
7	Apex	Acuminate to acute
8	Base	Cuneate to acute
9	Petiole	Short and sturdy
10	Margin	Entire
11	Venation	Reticulate
12	Texture	Glabrous
13	Length	6-12 cm
14	Width	2-5 cm
15	Petiole length	3 cm

Microscopical study

Microscopic examination of *Artabotrys hexapetalus* leaf showed the following characteristics using 0.8% safranin and 0.5% Astra blue and viewed under microscope of lens 45x.

Epidermis

T.S of the leaf is dorsiventral and passes through the midrib and lamina. The midrib is prominently projecting on the abaxial side and slightly convex on the adaxial side. Both surfaces possess a single-layered epidermis composed of compact, rectangular to oval cells covered by a thin cuticle. The mesophyll is differentiated into palisade and spongy tissues. A single layer of elongated palisade parenchyma occurs beneath the upper epidermis, interrupted by a few secretory cells. The spongy parenchyma consists of loosely arranged cells with conspicuous intercellular spaces towards the lower side.

Midrib region

It consists of several layers of parenchymatous ground tissue. A few layers of collenchymatous cells occur beneath the epidermis, providing mechanical support. A single, large collateral vascular bundle occupies the centre of the midrib. The vascular bundle is conjoint, collateral and closed, with the xylem oriented towards the adaxial side and the phloem towards the abaxial side. The vascular bundle is enclosed by a distinct parenchymatous bundle sheath and is partially surrounded by sclerenchymatous fibres.

Lamina

The lateral veins in the lamina contain smaller collateral vascular bundles enclosed by distinct bundle sheath cells. The ground tissue surrounding the vascular bundle consists mainly of thin-walled parenchymatous cells, some of which contain prominent cellular contents.

Petiole

T.S. is nearly circular to broadly semicircular, with a slightly undulating margin and shallow adaxial groove. Epidermis is single-layered with thin cuticle, followed by 2–5 layers of collenchymatous hypodermis. Ground tissue is parenchymatous with numerous secretory cells. Three prominent collateral vascular bundles occur in an arc, with smaller trace bundles. Prismatic crystals are present.

Fig 2: TS of *Artabotrys hexapetalus* leaf passing through midrib

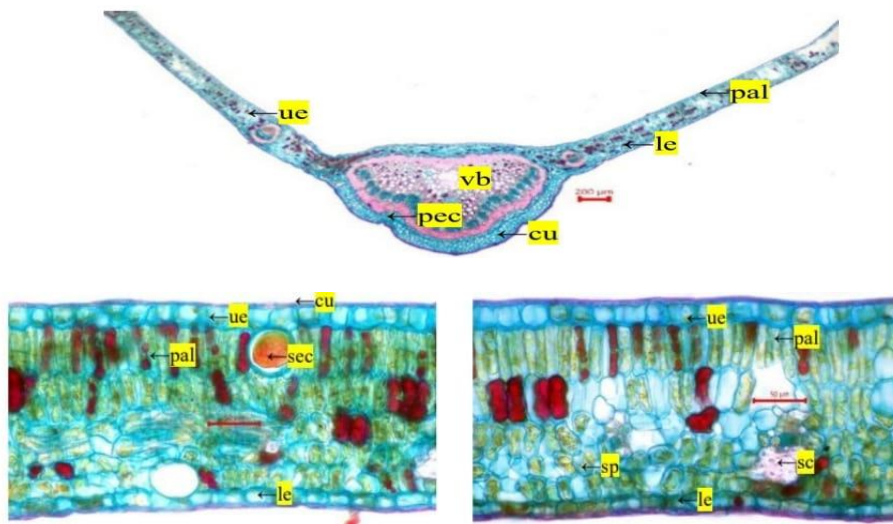


Fig 3: TS of margin

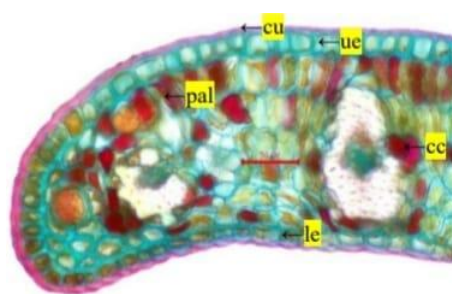


Fig 4: TS of lamina

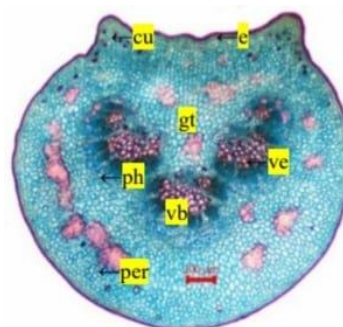


Fig 5: TS of petiole

Histochemical studies

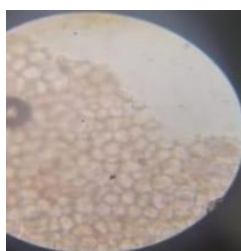
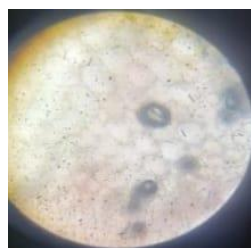
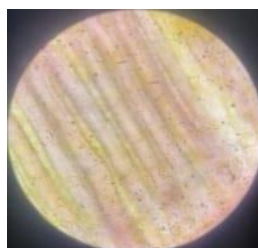
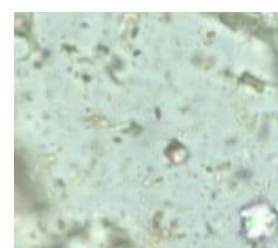
The section of the leaves of *Artabotrys hexapetalus*., were stained by using specific reagents such as N/50 Iodine, sulphuric acid, Phloroglucinol and Conc. Hydrochloric acid, KOH to observe as per the protocol.

Table 2: Histochemical studies of *Artabotrys hexapetalus* leaf

S.No.	Reagent	Test	Nature of change	Observation
1	Iodine solution followed by Sulphuric acid	Cellulose	Yellow	Parenchyma cells
2	Heating with KOH	Suberin	Yellow	stomata
3	Picric acid	Alkaloids	Yellow	Prismatic crystals
4	Phloroglucinol + Hcl	Lignin	Pink	Vessels

Fig 6: Histochemical studies of *Artabotrys hexapetalus* leaf**Quantitative microscopy of *Artabotrys hexapetalus* leaf****Determination of Phyto-constants of *Artabotrys hexapetalus* leaf**

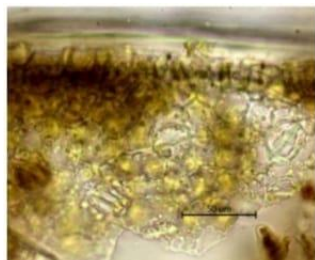
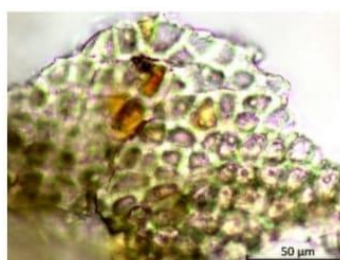
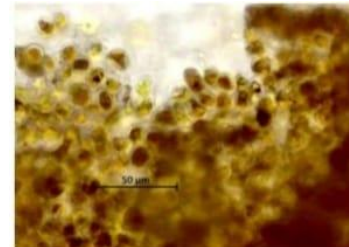
Leaf surface showed the presence of veins, vein islets, vein termination, epidermal number, stomatal number, stomatal index and palisade ratio were determined in upper epidermis and given in Tab.3

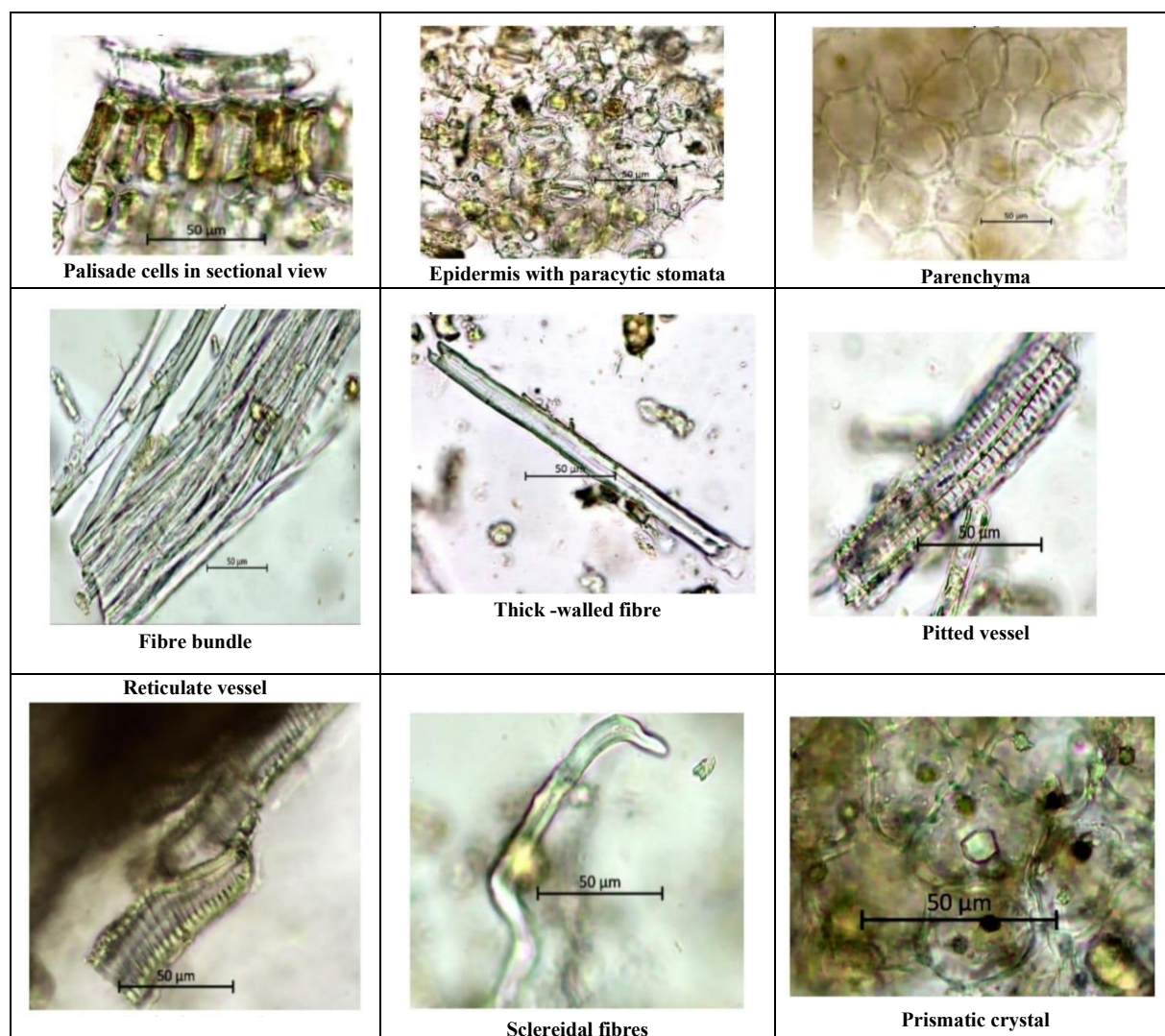
Table 3: Quantitative microscopy of *Artabotrys hexapetalus* leaf**Parenchyma cells****Stomata****Vessels****Prismatic crystals**

S.No.	Parameters	Cells /mm ²
1	Epidermal number	380-400
2	Vein islets number	35
3	Vein termination	40
4	Stomatal number	Upper epidermis: 30 Lower epidermis: 22
5	Stomatal Index	15
6	Palisade ratio	1-7

Powder microscopy

The shade dried, powdered plant material was used for powder microscopic analysis. The organoleptic characters were observed and to identify the different characteristic features various staining reagents are used. It showed epidermal cells, stomata of paracytic type, parenchyma cells, prismatic crystal.

**Sectional view of epidermis****Pericyclic stone cells****Mesophyll**

Fig 7: Powder microscopy of *Artabotrys hexapetalus* leaf**Behaviour of powder with various chemical reagents**

Powdered drug of plant gave different fluorescence under visible and ultraviolet (UV) radiation (254nm & 365nm) when treated with various reagents. The colour observed in different radiations were recorded. It is used for the identification of plant and powdered drug.

Table 4: Behavior of *A. hexapetalus* powder with various chemical reagents

S.No.	Reagent	Observation		
		Visible (<400 nm)	UV (254nm)	UV (365 nm)
1	Powder	Light green	Light green	Pale green
2	Powder+ water	Green	Dark green	Green
3	Powder+ conc HCl	Dark green	Brownish green	Dull green
4	Powder+ conc H ₂ SO ₄	Orange	Black	Reddish brown
5	Powder+ conc HNO ₃	Orange	Yellowish brown	Yellow
6	Powder+ CH ₃ COOH	Brown	Brown	Light green
7	Powder+ conc HCl+ H ₂ O	Dark green	Greenish brown	Green
8	Powder+ conc HNO ₃ + H ₂ O	Brown	Yellowish brown	Brown
9	Powder+ conc H ₂ SO ₄ + H ₂ O	Dark brown	Blackish brown	Reddish brown
10	Powder+ CH ₃ COOH+ H ₂ O	Green	Green	Bright green

11	Powder+ Aq KOH	Green	Dark green	Bright green
12	Powder+ Alcoholic KOH	Green	Greenish black	Yellowish green
13	Powder+ Aq FeCl ₃	Black	Black	Dull brown
14	Powder+ Alcoholic FeCl ₃	Black	Black	Brownish Black

Determination of physicochemical parameters & inorganic element analysis

The powdered drug of *Artabotrys hexapetalus* leaf was subjected to determine the physicochemical constituents and inorganic elements and heavy metals.

Table 5: Determination of Physicochemical Parameters and Inorganic elements and Heavy metal analysis of *Artabotrys hexapetalus* leaf

Preparation of hydroalcoholic extract of *Artabotrys hexapetalus* Leaf (HAEAL)

The collected leaves were washed with water, shade dried and powdered. 10 grams of power was extracted with hydroalcohol by maceration

S.No.	Physicochemical Parameters	Result
1	Foreign matter	Nil
2	Loss on drying	16.18±7.13 % w/w
3	Total solid	84 % w/w
4	Petroleum ether extractive	10.9±0.96 % w/w
5	Chloroform soluble extractive	36.7±0.56 % w/w
6	Ethyl acetate extractive	22.7±6.71 % w/w
7	Ethanol extractive	22 ±6.12 % w/w
8	Aqueous extractive	12.8±2.53 % w/w
9	Total ash	12.19±8.26 %w/w
10	Water soluble ash	5.65±1.22 %w/w
11	Acid insoluble ash	9.6±1.03 % w/w
12	Inorganic elements	Nil
13	Heavy metals – Lead	Absent

technique until the complete extraction of material and filtered. The filtrate was concentrated to dryness and percentage was found to be 23.34 % w/v. The pH was obtained as 6.0. The preliminary test was carried from this extract.

Qualitative phytochemical analysis

The extract were subjected to preliminary phytochemical screening to determine the presence of various phytoconstituents. It showed the presence of alkaloid, carbohydrates, steroids, phenol, tannins, saponins, flavonoid, terpenoids and proteins.

Table 6: Qualitative phytochemical analysis of *Artabotrys hexapetalus*

S.No.	Phytochemical analysis	Observation
1	Test for Alkaloids	
	Mayer's test	-
	Wagner's test	+
	Dragendroff's test	+
	Hager's test	+
2	Test for Tannins	
	With Fecl ₃	+
	With Gelatin	+
3	Test for Steroids	
	Salkowski test	+
	Liebermann-burchard test	+
4	Test for Terpenoids	
	Salkowski test	+
5	Test for Proteins	
	Biuret test	+
6	Test for Amino acids	
	Millon's test	+
	Ninhydrin test	+
	Wit Nitric acid	+
7	Test for carbohydrates	
	Benedicts test	+
	Molisch' test	+
	Fehling test	+
8	Test for Flavonoids	
	Shinoda test	+
	With Lead acetate	+
9	Test for mucilage	+
10	Test for triterpenoid	+
11	Test for gum	-

12	Test for diterpenoid	
	Copper acetate test	-
13	Test for anthocyanins	-
14	Test for Cardiac glycosides	
	Keller-killani test	-
	With Alcoholic FeCl ₃	-
	Legal's test	-
	Baljet's test	-
15	Test for Saponins	
	Foam test	-
16	Test for Anthroquinone glycosides	
	Modified Born-trager's test	-
17	Test for Quinone	
	Bamberger test	-
	Conc. Hcl	-
18	Test for coumarin	-

+ Presence, - absence

Quantitative estimation of phytoconstituents

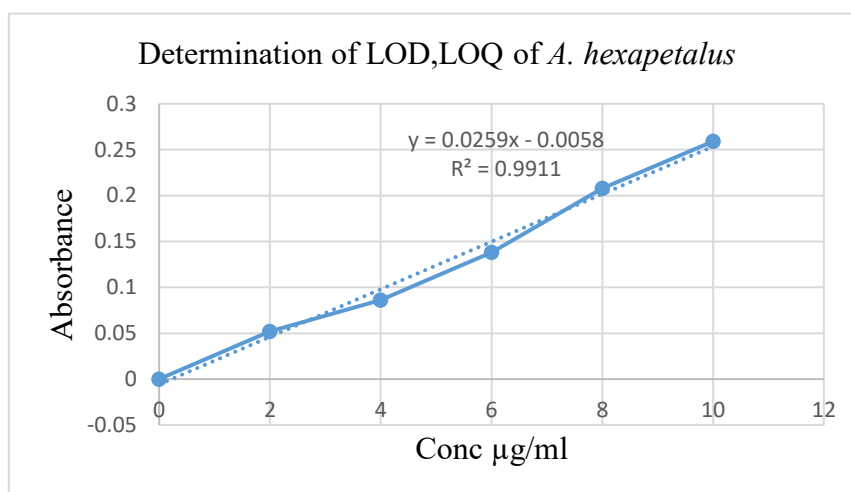
Quantitative analysis such as Total phenolic content, Total Tannin content, Total flavonoid content, were estimated for the *Artabotrys hexapetalus* extract using UV-Vis spectrophotometer.

Determination of LOD and LOQ of *Artabotrys hexapetalus*

Various concentrations (0 to 10 µg/ml) of *Artabotrys hexapetalus* were prepared and observed under UV at λ_{max} 340 nm. Absorbance was displayed in table 7.

Table 7: Determination of LOD and LOQ of *Artabotrys hexapetalus*

S. No	Concentration (µg/ml)	Absorbance
1	2	0.052
2	4	0.086
3	6	0.138
4	8	0.208
5	10	0.259
Limit of detection (LOD)		18.15 µg/ml
Limit of quantity (LOQ)		55 µg/ml

Figure 8: Determination of LOD and LOQ of *Artabotrys hexapetalus*

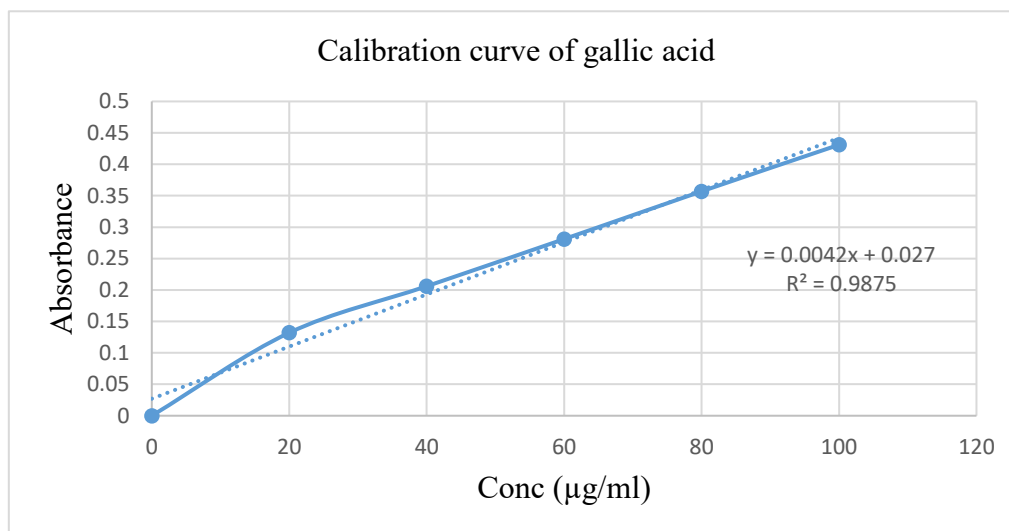
The quantitative estimation LOD and LOQ of *A. hexapetalus* was found to be 18.15 and 55 µg/ml.

Determination of gallic acid equivalent in *A. hexapetalus***Table 8: Determination of gallic acid equivalent in *A. hexapetalus***

S.No.	Concentration (µg/ml)	Absorbance of gallic acid	Absorbance of <i>A. hexapetalus</i>
1	0	0	0
2	20	0.132	0.192
3	40	0.206	0.216
4	60	0.281	0.240

5	80	0.357	0.263
6	100	0.431	0.286
		GAE	616.7mg/g

Figure 9: Calibration curve of gallic acid



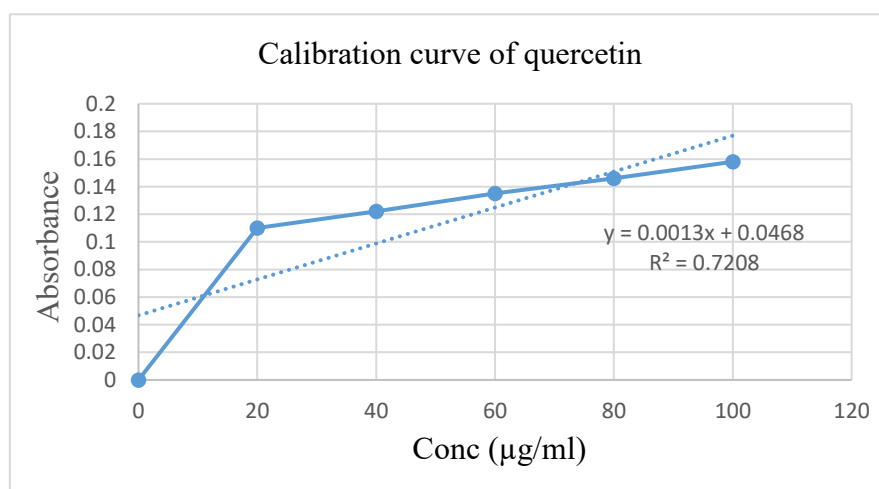
Quantitative estimation of phenolic content was performed using Folin Cio-Calteau method, with gallic acid as a standard. The total phenolic content was calculated using the following linear equation, derived from the calibration curve of standard gallic acid, $Y = 0.0042x + 0.027$ with a correlation coefficient $R^2 = 0.9875$. The total phenolic content was expressed in terms of GAE. The total phenolic content in *Artabotrys hexapetalus* was found to be 616.7 mg GAE/g of dried sample.

Determination of Quercetin Equivalent in *Artabotrys hexapetalus*

Table 9: Determination of Quercetin equivalent in *A. hexapetalus*

S.No.	Concentration (µg/ml)	Absorbance of Quercetin	Absorbance of <i>A. hexapetalus</i>
1	0	0	0
2	20	0.110±0.0020	0.037±0.0005
3	40	0.122±0.0014	0.057±0.0004
4	60	0.135±0.0004	0.076±0.0010
5	80	0.146±0.0001	0.096±0.0003
6	100	0.158±0.0006	0.118±0.0012
		QE	550 mg/g

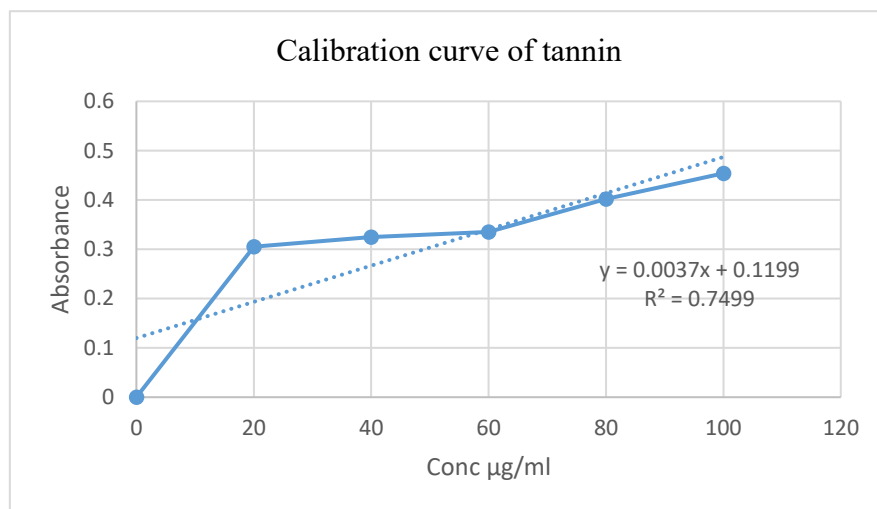
Figure 10: Calibration curve of Quercetin



Quantitative estimation of flavonoid content was performed using Aluminium chloride colorimetric assay method, with quercetin as a standard. The total flavonoid content was calculated using the following linear equation, derived from the calibration curve of standard quercetin, $Y = 0.0013x + 0.0468$ with a correlation coefficient $R^2 = 0.7208$. The total flavonoid content was expressed in terms of QE. The Total flavonoid content in *Artabotrys hexapetalus* was found to be 550 mg QE/g of dried sample.

Determination of Tannic acid Equivalent in *Artabotrys hexapetalus***Table 10: Determination of Tannic acid equivalent in *A. hexapetalus***

S.No.	Concentration (µg/ml)	Absorbance of tannic acid	Absorbance of <i>A. hexapetalus</i>
1	0	0	0
2	20	0.305±0.0001	0.176±0.0012
3	40	0.325±0.0003	0.245±0.0004
4	60	0.335±0.0005	0.271±0.0001
5	80	0.402±0.0002	0.297±0.0009
6	100	0.454±0.0001	0.311±0.0002
		TAE	520 mg/g

Fig 11: Calibration curve of tannic acid

Quantitative estimation of Tannin content was performed using Folin-Denis method, with Tannic acid as a standard. The total tannin content was calculated using the following linear equation, derived from the calibration curve of standard tannic acid, $Y = 0.0037x + 0.1199$ with a correlation coefficient $R^2 = 0.7499$. The total tannin content was expressed in terms of TAE. The Total tannin content in *Artabotrys hexapetalus* was found to be 520 mg TAE/g of dried sample.

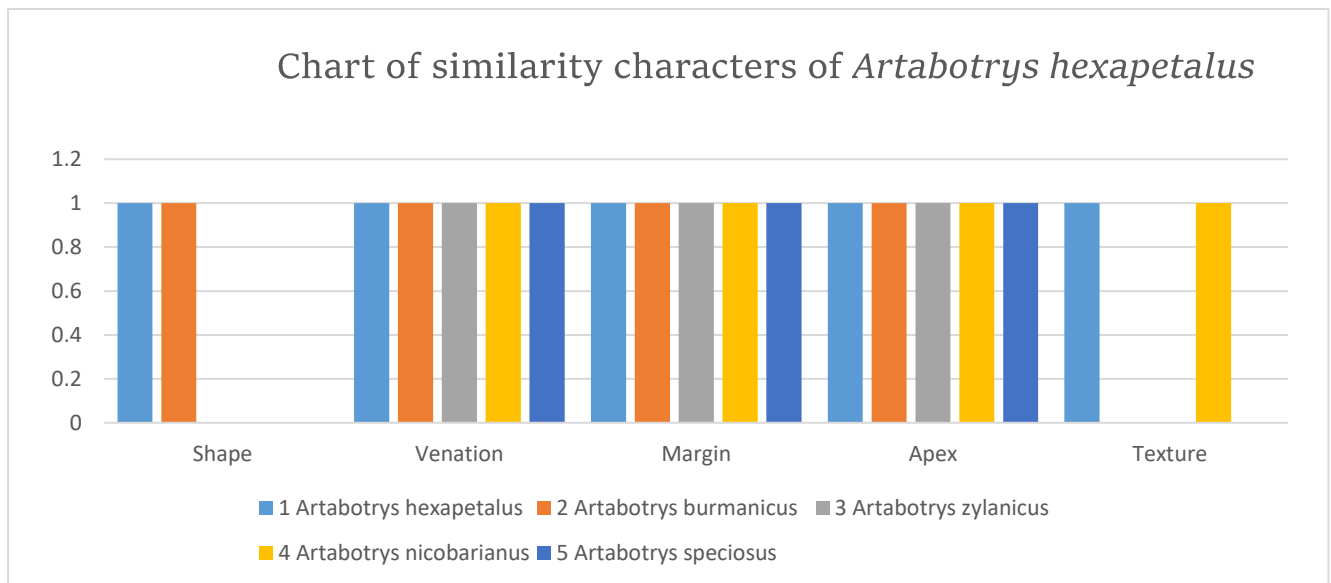
Phenetics

The *Artabotrys* genus from the family of Annonaceae, includes about 110 species are found in all over India. Among those 5 different species are found in India. The following six species of *Artabotrys* was selected for numerical taxonomy.

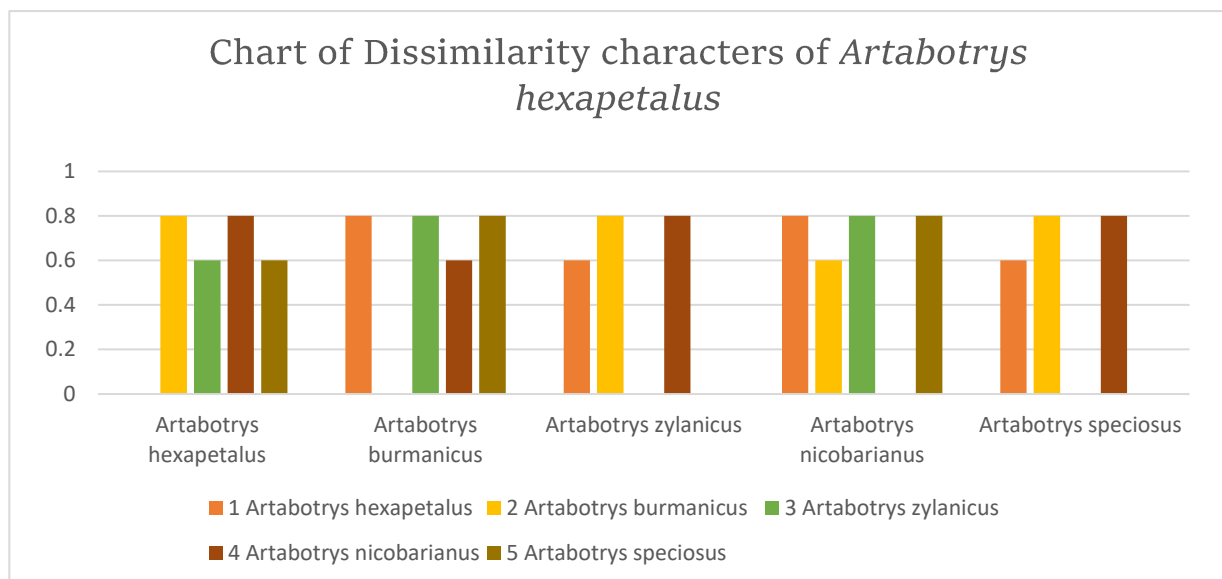
1. *Artabotrys hexapetalus*
2. *Artabotrys burmanicus*
3. *Artabotrys zylanicus*
4. *Artabotrys nicobarianus*
5. *Artabotrys speciosus*

Table 11: Chart of similarity characters of *Artabotrys hexapetalus*

S.No	Species	Shape	Venation	Margin	Apex	Texture
1	<i>Artabotrys hexapetalus</i>	1	1	1	1	1
2	<i>Artabotrys burmanicus</i>	1	1	1	1	0
3	<i>Artabotrys zylanicus</i>	0	1	1	1	0
4	<i>Artabotrys nicobarianus</i>	0	1	1	1	1
5	<i>Artabotrys speciosus</i>	0	1	1	1	0

Figure 12: Chart of similarity characters of *Artabotrys hexapetalus*Table 12: Chart of Dissimilarity characters of *Artabotrys hexapetalus*

S.No	Species	<i>Artabotrys hexapetalus</i>	<i>Artabotrys burmanicus</i>	<i>Artabotrys zylanicus</i>	<i>Artabotrys nicobarianus</i>	<i>Artabotrys speciosus</i>
1	<i>Artabotrys hexapetalus</i>	0	0.8	0.6	0.8	0.6
2	<i>Artabotrys burmanicus</i>	0.8	0	0.8	0.6	0.8
3	<i>Artabotrys zylanicus</i>	0.6	0.8	0	0.8	0
4	<i>Artabotrys nicobarianus</i>	0.8	0.6	0.8	0	0.8
5	<i>Artabotrys speciosus</i>	0.6	0.8	0	0.8	0

Figure 13: Chart of Dissimilarity characters of *Artabotrys hexapetalus***Sample Matching Coefficient**

$$SSM = NS / (NS + ND) * 100$$

Where, NS – Number of Similarity characters

ND – Number of Dissimilarity characters

Number of similar characters = 38

Number of Dissimilar characters = 12

The matching coefficient of *A. hexapetalus* with respect to other species was found to be 76%.

Conclusion

The present study establishes comprehensive pharmacognostical and phytochemical standards for *Artabotrys hexapetalus* leaves sourced from Ethappur village, Salem district, Tamil Nadu. Macroscopic, microscopic and powder characters including dorsiventral lamina, paracytic stomata, prismatic crystals, pericyclic stone cells and defined leaf constants (vein islet number- 35, vein termination 40, stomatal index 15, palisade ratio 1–7) together with fluorescence behaviour and physicochemical values serve as reliable diagnostic markers for authentication. The absence of lead confirms the safety of the drug with respect to heavy metal contamination. Preliminary screening of the hydroalcoholic extract revealed alkaloids, carbohydrates, proteins, steroids, terpenoids, phenols, tannins and flavonoids, while quantitative estimation showed appreciable total phenolic (616.7 mg GAE/g), flavonoid (550 mg QE/g) and tannin (520 mg TAE/g) contents, which may account for the reported pharmacological activities of the plant. Phenetic analysis yielded a matching coefficient of 76% with related *Artabotrys* species, indicating close vegetative affinity within the genus. These findings provide useful reference standards for the identification and quality control of *A. hexapetalus* leaf and form a basis for further isolation of its bioactive constituents.

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REFERENCE :

1. Kousalya.P, Doss. A review on *Artabotrys hexapetalus* (L.f.) Bhandari: Ethnomedicinal uses, pharmacological properties and bioactive compounds. Journal of huazhong university of science and technology. 2021;50(7): 1-11.
2. Ranganathan R, Vijayalakshmi R, Parameswari P. Ethnomedicinal plants and their utilization by villagers in Jawadhu hills of Thiruvannamalai district of Tamil Nadu, India. Inte J Pharm Res Dev 2012b; 4(4): 174–183.
3. Hout S, Chea A, Bun SS, Elias R, Gasquet M, Timon-David P, et al. Screening of selected indigenous plants of Cambodia for antiplasmodial activity. J Ethnopharmacol 2006; 107(1): 12–18.
4. Jayanthi CK. Phyto-pharmacognostic and experimental study of *Artabotrys hexapetalus* (Linn.f.) Bhandari w.s.r to its antidiarrhoeal and anthelmintic effect. MD thesis, Rajiv Gandhi University of Health Sciences 2011.
5. Rajkumar MH, Rajanna MD. Ex-situ conservation of climbing plants at University of Agricultural Sciences, Bangalore, Karnataka. Recent Res Sci Technol 2011; 3(4): 18–20.
6. Karthik YP. Evaluation of antifertility activity of leaves of *Artabotrys hexapetalus*. MPharm thesis, Rajiv Gandhi University of Health Sciences 2010;1-11.
7. Savadi RV. Phytochemical investigations and antifertility properties of some medicinal plants. PhD thesis, Rajiv Gandhi University of Health Sciences 2009;1-11.
8. Zheng XL, Xing FW. Ethnobotanical study on medicinal plants around Mt. Yinggeling, Hainan Island, China. J Ethnopharmacol 2009; 124(2): 197–210.
9. Hedberg I, Hedberg O, Madati PJ, Mshigeni KE, Mshiu EN, Samuelsson G. Inventory of plants used in traditional medicine in Tanzania. I. Plants of the families Acanthaceae, Cucurbitaceae. J Ethnopharmacol 1982; 6(1): 29–60.
10. Chuakul W, Soonthornchareonnon N. Ethnomedical uses of Thai Annonaceous plant (1). Thai J Phytopharm 2003; 10(1): 25–32.
11. K.M. Sowjanya, J. Swathi, K. Narendra, CH. Padmavathi, A. Krishna satya. Extraction and Antimicrobial potential of secondary plant metabolites from *Artabotrys hexapetalus* (Linn.f) Bhandari. International Journal of Research in Ayurveda and Pharmacy. 2013;4(5):764-768.
12. Christian Bailly, Jean-Pierre Henichart. A Review Advocacy for the Medicinal Plant *Artabotrys hexapetalus* (Yingzhao) and Antimalarial Yingzhao Endoperoxides. 2022;27 ,6192:1-17.
13. Rahini D and Anuradha R. In-vitro antioxidant activity of *Artabotrys hexapetalus*. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2014;5(2): 396-405.
14. Manjula M, Kumuda KV, Anitha S, Shashidhara S. Antioxidant and antimicrobial activities of various extracts of *Artabotrys hexapetalus* flowers. An International Journal of Pharmaceutical Sciences. 2011;2(3):42-50.
15. Veena Rani I, Annapurna A, Ganapathi S. Evaluation of Hepatoprotective activity and Oxidative stress parameters of alcoholic extract of *Artabotrys hexapetalus* (L.f) Bhandari. Journal of Global Trends in Pharmaceutical Sciences. 2016;7(2):3192-3199.
16. Md. Akhlakur Rahman et al. Antioxidant, analgesic, anti-inflammatory and antipyretic potentialities of leaves of *Artabotrys hexapetalus*, established by in silico analysis. Phytomedicine Plus 3. (2023);1-12.
17. Niaz Morshed, Md. Mizanur Rahman Moghal, Mohammad Nurul Amin. Investigation of in-vitro anthelmintic and cytotoxic activities of *Artabotrys hexapetalus* (family: annonaceae) bark growing in Bangladesh. Trends in Biotechnology Research. (2012);1(2):27-30.
18. Mohana Krishnan.P, Sandhiya.V, Shahina.A, Mahalakshmi.A, Vigneshwaran.L.V. *Artabotrys hexapetalus*: Integrating Numerical taxonomy with Pharmacognostical, Phytochemical and Pharmacological insights. International Journal of Research in Pharmacology &

- Pharmacotherapeutics. 2025;14(3):608-618.
19. Bajaj Sakshi , Wakode Sharad. Standarization of *Artabotrys hexapetalus*. International Journal of Pharmacognosy and Phytochemical Research 2016; 8(3); 398-406.
 20. Wallis TE. Analytical microscopy: its aims and methods in relation to foods, water, spices and drugs. 3rd ed. Boston: Little, Brown and Company; 1965.
 21. Khandelwal KR. Practical Pharmacognosy: Techniques and Experiments. 19th ed. Pune: Nirali Prakashan; 2008.
 22. Kokoshi CJ and Kokoshi RJ, Sharma PJ. Fluoresence of powder vegetables drugs under UV radiation. J Am. Pharm.Assoc. 47,1958,715-717.
 23. Anonymous, The Ayurvedic pharmacopoeia of India. Edn 1, vol. 2, Government of India, New Delhi,1986,pp.189-196.
 24. Bentley and Driver's Text book of pharmaceutical chemistry. Edn.8. L.M.Atherden, 1961:pp.174-176.
 25. Indian Pharmacopoeia. 3rd ed. New Delhi: Ministry of Health and Family Welfare, Government of India, Controller of Publications; 1985:245
 26. J.B.Harbone. Phytochemical methods A guide to modern techniques of plant analysis. Edn 3,1998.
 27. Seo, Ji-Hye, Jung-Eun Kim, Jung-Hyun Shim, Goo Yoon, Mi-Ae Bang, Chun-Sik Bae, Kyung-Jin Lee, Dae-Hun Park and Seung-Sik Cho, HPLC analysis, optimization of Extraction conditions and biological evaluation of *Corylopsis coreana* Uyeki Flos. Molecules.2016; 21(1): 94.
 28. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total Phenols and other oxidation substances and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol.1999;299:152-178.
 29. Boham BA, Kocipai-Abyazan R. Flavonoids and condensed tannins from leaves of *Hawaiian Vaccinium vaticulatum* and *V.calycinum*. Pac Sci.1994;48(4):458-63.
 30. Ashwini Kumar and Baidyanath Kumar2016.Biosystematic Studies in *Heliotropium indicum*, *Trichodesma indicum* and *T. zeylanicum* of Boraginaceae. Int.J.Curr.Microbiol.App.Sci.5(4),2016,720